Purification and Characterization of Helicobacter mustelae Urease

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Helicobacter mustelae is a urease-rich bacterium associated with gastritis in ferrets. The ureases of H. mustelae and Helicobacter pylori, a bacterium implicated in human gastritis, share many characteristics. Helicobacter sp. ureases appear to be unique among bacterial enzymes in exhibiting submillimolar Km values and in being composed of two subunits.

Helicobacter mustelae is a gram-negative, spiral, microaerophilic bacterium associated with gastritis in domestic ferrets (8, 24). Essentially all adult, but not pre-weanling (age, <6 weeks), ferrets are colonized with H. mustelae (7, 8). In addition, previously uninfected ferrets can be infected experimentally by oral challenge with H. mustelae (7, 9). The purpose of this study was to purify and characterize the urease of H. mustelae in order to compare it with the urease of Helicobacter pylori. The latter bacterium, known previously as Campylobacter pylori (10), has been implicated as an etiologic agent of gastritis and as a permissive factor in the pathogenesis of peptic ulcer disease in humans (2, 15, 21).

All strains of H. mustelae used were identified, passaged, and stored as described previously (7, 8). Cultures were grown for 72 h on Trypticase soy agar containing 5% sheep blood (Remel, Lenexa, Kans.) at 37°C in an atmosphere containing 5% O2, 7.5% H2, 7.5% CO2, and 80% N2. Cells from 60 plates (100 by 15 mm) with confluent growth were harvested into ice-cold 0.15 M NaCl. Bacterial cells were harvested by centrifugation (5,000 × g, 20 min, 4°C). Cells were suspended in 100 ml of distilled water, the suspension was vortexed for 45 to 60 s, and then cells were sedimented by centrifugation (10,000 × g, 20 min, 4°C). The supernatant was concentrated as described previously (5).

Urease activity was assayed by using an NADH-coupled enzyme assay (5). One unit of urease activity was defined as that amount capable of hydrolyzing 1 μmol of urea per min. The specific activity of urease was calculated as micromoles of urea hydrolyzed per minute per milligram of protein.

Urease was purified by subjecting water extracts sequentially to size-exclusion chromatography using an HR 16/50 column containing preparative-grade Superose 12 (Pharmacia LKB Biotechnology Inc., Houston, Tex.) and anion-exchange high-performance liquid chromatography using a DEAE 5PW Sphero gel TSK-IXE column (75 mm by 7.5 mm; Beckman Instruments, San Ramon, Calif.) by methods described previously (5). Fractions with enzyme activity greater than 250 units/ml were pooled. Ammonium sulfate (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1.7 M. Pooled fractions were applied to a Phenyl Superose HR 5/5 column (Pharmacia LKB) in buffer containing 20 mM potassium phosphate, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 1.7 M ammonium sulfate, pH 7.0, at a flow rate of 0.5 ml/min. Proteins were eluted with a decreasing linear gradient from 1.7 M to 0 M ammonium sulfate in the same buffer over 60 min. The molecular size of purified urease was determined by using a Superose 6 HR 10/30 column (Pharmacia LKB) in PEB buffer (5) containing 0.15 M NaCl at a flow rate of 0.5 ml/min by using methods described previously (5). Other analytical procedures were performed as described previously (5).

Results of a typical purification regimen are summarized in Table 1. Urease was purified 42-fold with an overall recovery of 27%. On the basis of 42-fold purification to homogeneity of enzyme activity from whole cells, urease constituted approximately 2% of the protein of H. mustelae 4634. Purified urease showed bands at 62 and 27.5 kDa upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (Fig. 1). The size of native H. mustelae urease deduced by size-exclusion chromatography was 335 kDa. The ratio of integrated densities of gel scan profiles of the 27.5-kDa subunit versus the 62-kDa subunit corrected for apparent molecular sizes was approximately 1.1. Thus, in native H. mustelae urease the calculated stoichiometry was (62 kDa-27.5 kDa)2. The apparent Km was 0.45 ± 0.1 mM. Under saturation conditions (100 mM urea), the specific activity of freshly purified urease was 1,560 ± 100 μmol of urea hydrolyzed per min per mg of protein.

The sequence of the N-terminal 17 amino acids of the 62-kDa subunit of H. mustelae urease was 94% identical to that of the 62-kDa subunit of H. pylori urease. In H. mustelae, isoleucine replaced lysine present in position 2 in H. pylori (5). The sequence of the N-terminal 20 residues of the 27.5-kDa subunit was 90% identical to that of the 30-kDa subunit of H. pylori urease. In H. mustelae, arginine and methionine replaced lysine and leucine present in positions 2 and 11, respectively, in H. pylori (5).

These results demonstrate striking similarities between the ureases of H. mustelae and H. pylori. For example, 65 to 80% of the urease activity of both species can be extracted in a single water wash step. This observation suggests that urease is expressed as a surface layer protein in Helicobacter species (5). In support of this hypothesis, monoclonal antibodies against urease bind to the outer membrane of intact H. pylori and have been used to radioimmunoprecipitate urease from H. pylori (20). Histochemical studies have demonstrated that urease is associated with the outer membrane and periplasmic space of H. pylori (3). Using an immunogold technique to localize urease, Hawtin et al. (11) demonstrated that the enzyme is located on the surface of H. pylori and in material apparently shed from that surface. In addition, radioiodination of intact H. pylori using immobi-
lized Iodogen has demonstrated that the 62-kDa subunit of urease is surface exposed (6). Taken together, these data suggest that urease is a major surface-related protein of H. pylori. We are not aware of similar studies to localize urease within H. mustelae.

Ureases of H. mustelae and H. pylori are similar in subunit composition and size of native enzyme. The urease of H. mustelae is composed of two subunits (62 kDa and 27.5 kDa) of sizes similar to those of H. pylori urease (subunit sizes, 62 to 66 kDa and 29.5 to 30 kDa [5, 13]). The size of native urease of H. mustelae (535 kDa) is similar to that of H. pylori (550 kDa [13]). In three separate but related analyses, we have redetermined the size of purified native urease of H. pylori 84-183 to be 548 kDa, not 380 kDa as was reported previously (5). This new determination, based on the use of a Superose 6 column, with superior resolution of large proteins compared with the Superose 12 column used previously (5), is in excellent agreement with the results of Hu and Mobley (13). The apparent stoichiometry of native H. mustelae urease ([62 kDa-27.5 kDa]3) is identical to that of native H. pylori urease (13). Furthermore, the K_m observed for purified H. mustelae urease (0.45 mM) is similar to that of H. pylori urease (0.2 to 0.3 mM [5, 13]). The low K_m of Helicobacter sp. ureases presumably reflects an adaptation to low gastric urea concentrations (5, 17).

Urease activity appears to be an important virulence factor in H. pylori and presumably contributes to the pathogenesis of H. mustelae as well. Urease activity generates ammonium from urea in the immediate bacterial microenvironment, thus protecting H. pylori from the deleterious effects of gastric acid (16, 18). In addition, urease activity may inhibit the biosynthesis of mucus and/or cause gastric mucus to be disassembled at the mucosal surface, changes which may facilitate colonization by H. pylori and possibly promote formation of peptic ulcers (22). Furthermore, high concentrations of ammonium ion resulting from urease activity may exert toxic effects upon intercellular junctions, resulting in alteration of gastric mucosa permeability (12). In vitro, H. pylori urease activity exerts toxic effects on cultured epithelial cells and on human gastric mucus-secreting cells (1, 23). Cytotoxicity is increased significantly in the presence of exogenous urea and is inhibited by urease inhibitors (23). Thus, the cytotoxic effects of urease activity are thought to be mediated directly by ammonium ions, not by indirect effects such as changes in pH of the culture medium (1, 23).

A novel feature of Helicobacter sp. ureases is the presence of two polypeptides in the active enzyme (4, 5, 13, 14). In contrast, other well-characterized bacterial ureases are composed of either one or three subunits (5, 17). The significance of the differences in the number of subunits in bacterial ureases is not known.

Taken together, our data demonstrate significant similarities between the ureases of H. mustelae and H. pylori. These similarities are in keeping with the recognized morphologic, biochemical, and genetic similarities between these two Helicobacter species (9, 10, 19). Such similarities support the hypothesis that infection of ferrets with H. mustelae will serve as a useful model of human infection with H. pylori (7, 9).

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REFERENCES


<table>
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<tr>
<th>Purification</th>
<th>Sp act (µmol of urea/min/mg of protein)</th>
<th>Purification (fold)</th>
<th>Total activity (µmol/min)</th>
<th>Enzyme recovery (%)</th>
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FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified H. mustelae urease. Lane contains 0.10 unit of enzyme activity after Phenyl Superose chromatography. Bands were visualized by silver staining. The positions and sizes (in kilodaltons) of molecular size standards are shown at the left of the figure.